

# Determination of Phenols in Environmental Matrices using HPLC with an Enzyme Electrode Detector

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## 1. ABSTRACT

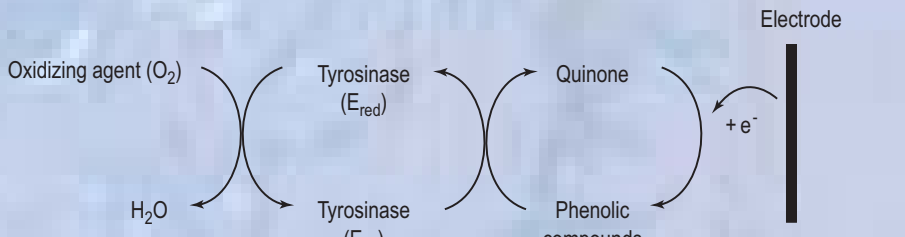
A simple and rapid assay using HPLC with a tyrosinase-containing carbon paste electrode (Tyr-CPE) detector is demonstrated for the detection of phenol, p-cresol, p-methoxyphenol, and p-chlorophenol in environmental matrices. These compounds were measured in contaminated aqueous soil leachate samples or acetonitrile/acetone extracts of soil and sludge samples. The dynamic range for this assay was 2 ppm to 30 ppm for phenol and p-cresol. In addition to analysis by the Tyr-CPE detector held at a reducing potential (-0.20V), soil leachate and extracts were analyzed using a carbon paste electrode (CPE) detector at oxidizing potentials (+1.0V or +1.2V vs. Ag/AgCl). Compared to the CPE, the Tyr-CPE detector was very stable, showing a signal loss of only 28% over 24 hr of continuous operation. In addition, the signal from the CPE degraded rapidly by 65% over ten sequential injections while the signal of the Tyr-CPE decreased by only 5% for the same number of sequential injections. Due to the minimal sample preparation for analysis of soil leachate samples, the herein reported chromatographic system with biosensor detector could be further developed to complement portable tyrosinase enzyme electrode biosensors that detect total phenols in field settings.

## 2. INTRODUCTION AND OBJECTIVES

There is considerable interest for the determination of phenolic compounds in environmental matrices because of their toxicity (1). These compounds have a diversity of sources (e.g. herbicides, insecticides, disinfectants, and their metabolites) and may contaminate the environment as a result of a number of manufacturing, refining, and agricultural processes. Analytical methods for measurement of phenols in environmental matrices include colorimetry, gas chromatography, liquid chromatography, and mass spectrometry. Although the chromatographic techniques are sensitive and specific, they are also typically complex, expensive, and time-consuming. With respect to potential field screening applications, electrochemical sensors and detectors are extremely attractive for monitoring phenolic compounds. Although the phenol moiety can be directly oxidized at moderate potentials (ca. +1.0V vs. Ag/AgCl reference), opening the way for direct anodic detection of these compounds, a biosensor in which the enzymatic activity of tyrosinase (polyphenol oxidase, EC 1.14.18.1) is coupled with amperometric transduction yields certain advantages over classical amperometric methods. Because the operation of these biosensors relies on the enzymatic conversion of the phenolic substrates to quinones, these compounds can be measured at small negative potentials (e.g., -0.05 to -0.2V) by reductive detection of the quinone product.

Tyrosinase is a copper containing oxidase which catalyzes the reduction of molecular oxygen to water by a variety of electron donors, as is demonstrated for phenolic compounds, in the following Scheme. At first, a dihydroxy derivative is generated by a two-electron process followed by further oxidation to the corresponding quinone. The resulting quinone product is electrochemically reduced by the electrode.

The tyrosinase enzyme electrode has been demonstrated as a portable/remote detector for phenol and p-cresol in ground water. The previously reported biosensor technique, however, was not able to differentiate between various phenols. This report demonstrates the application of HPLC using enzyme and nonenzyme-based electrochemical detection to determine specific phenols in contaminated soil leachates and extracts of soil and sludge (2).



## 3. METHODS

**Environmental Samples.** Environmental leachate samples were obtained from, U.S. EPA Region III (treatment plant influent from the EDM site, referred to as Leachate 1), Region II (treatment plant influent from the Oxy-Chem site, referred to as Leachate 3) and Region IV (treatment effluent from the Data Chem site, referred to as Leachate 2). Contaminated sludge was from Region IV (Data Chem site).

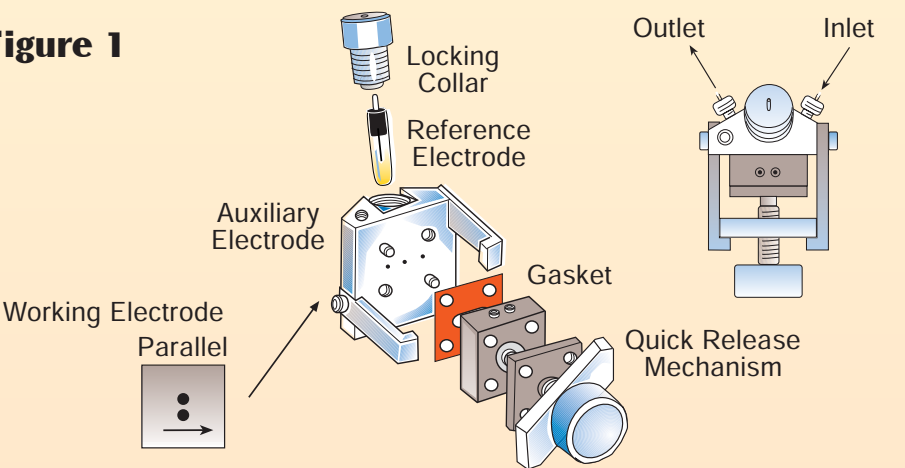
**Figure 1. High Performance Liquid Chromatography.** All standard solutions except where specified were prepared in the mobile phase. All mobile phases, leachate samples, and solutions were filtered and degassed prior to use. All experiments were performed on a reverse phase high performance liquid chromatography system (BAS, Model 480) equipped with a dual carbon paste thin layer flow cell. Injections were made manually using a 20  $\mu$ L loop (Reodyne injection valve), onto a C<sub>18</sub> column (BAS, MF6213, ODS-3, 3  $\mu$ m, 3.2 x100 mm) using a flow rate of 0.5 mL/min. Chromatograms were recorded using an Omniscribe strip chart dual pen recorder (Houston Instruments) or were processed using BAS Chromgraph software.

**Electrode Design and Preparation.** For dual electrode experiments using the thin layer cell (BAS MF-1004), each electrode was operated independently in a separate run. One cavity contained the CPE prepared using a graphite powder/mineral oil composition (60/40), which was mixed thoroughly, and then packed into the electrode cavity. The second cavity was used for the enzyme electrode (Tyr-CPE) and was prepared using carbon paste, with a tyrosinase content of 3% (w/w).

**Sample Preparation.** Soil and sludge samples received from EPA Regional Offices were extracted by using EPA method 3540. For soil samples, secondary stock solution concentrations were adjusted to allow the combination of 5 mL with 20 g soil. After thorough mixing, the acetone was evaporated under N<sub>2</sub> prior to extraction of the spiked soils.

### Flow Cell Detector

Figure 1



## 4. RESULTS

One of the significant advantages of the presently described technique over many laboratory methods is the minimal sample preparation required. Sample preparation for aqueous leachate samples analyzed by the herein reported technique included neutralization with KOH (because the samples were stabilized for shipment with H<sub>2</sub>SO<sub>4</sub>) and passage through a 0.2  $\mu$ m filter to remove particulates. This minimal sample preparation makes this technique attractive for potential development as a field analytical method using a portable HPLC or a low pressure flow injection analysis system.

**Figure 2. Chromatograms and calibration plots obtained using the Tyr-CPE detector for phenol and p-cresol.** Calibration plots for both phenol (peaks and plot labeled 1) and p-cresol (peaks and plot labeled 2) showed linear relationships for peak current responses between 2 ppm and 30 ppm. Phenols were diluted into mobile phase at final concentrations of (a) 2 ppm, (b) 5 ppm, (c) 10 ppm, (d) 20 ppm, and (e) 30 ppm. The tracings show sequential injections and arrows indicate the injection point. Applied potential was -0.2V (vs. Ag/AgCl), the mobile phase was acetonitrile/PBS (25/75), and the flow rate was 0.5 mL/min.

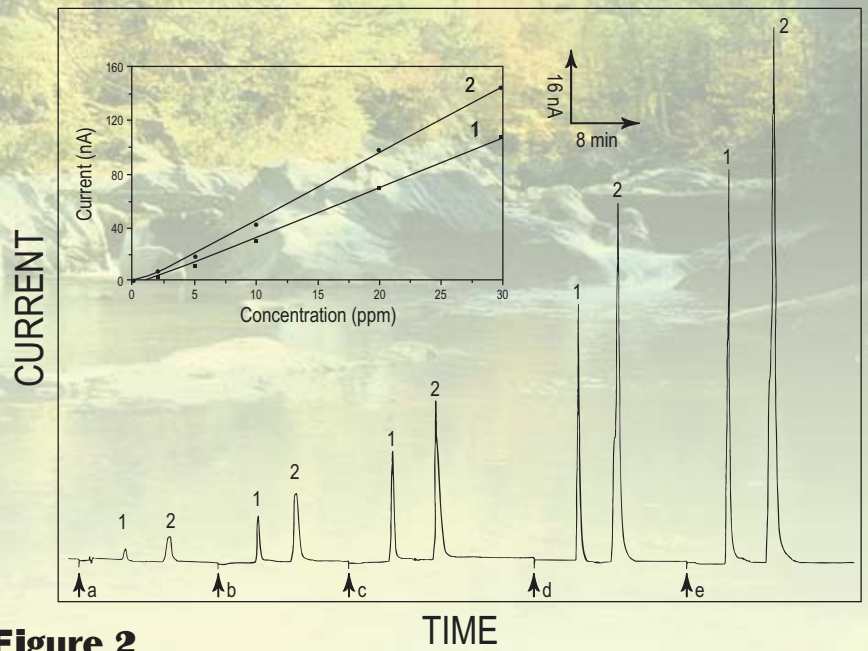


Figure 2

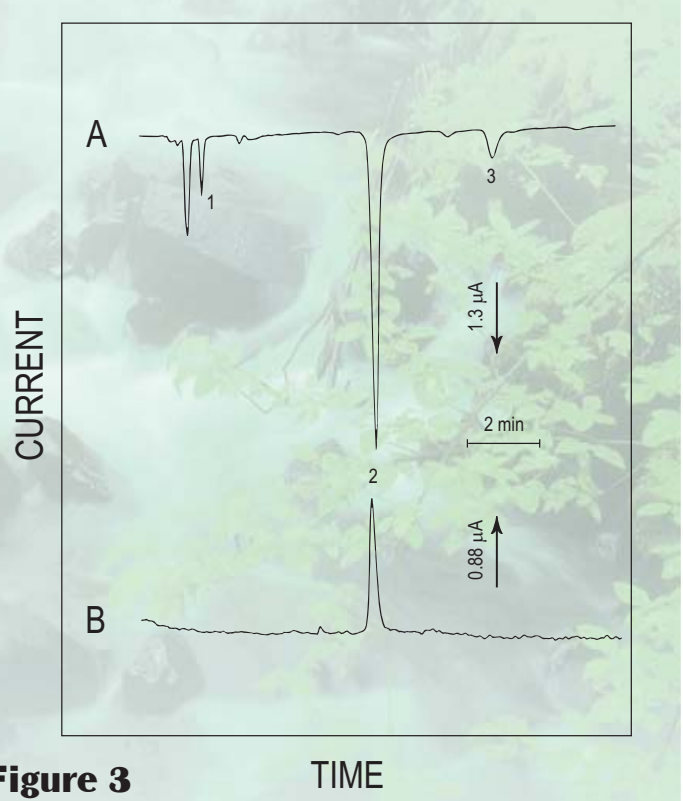


Figure 3

**Figure 3. Dual detector chromatograms obtained using CPE (A) or Tyr-CPE (B) detectors for Leachate 3.** Peaks were identified as p-acetamidophenol (peak 1), phenol (peak 2), and, p-nitrophenol (peak 3). The Tyr-CPE detector resulted only in a peak corresponding to phenol. Applied potentials were +1.0V for the CPE (A) and -0.2V for the Tyr-CPE (B). As indicated by the current arrows, chromatogram A is inverted. Other conditions as in Fig. 2.

**Figure 4. Dual electrode chromatograms for extracts of soil which had been spiked with o-nitrophenol, phenol, o-cresol, p-cresol, 2,4-dinitrophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, pentachlorophenol, and p-nitrophenol, each at 1.2 ppm.** The simplicity of the Tyr-CPE response arises from the enzyme's selectivity for p-cresol and phenol. This enzyme selectivity has also been observed for several tyrosinase electrode configurations. Potentials applied to the CPE (chromatograms, A) and Tyr-CPE (chromatograms, B) as well as other conditions were as in Fig. 3. Peaks for the Tyr-CPE detector were identified as phenol (peak 1) and p-cresol (peak 2). The tracings show sequential injections indicated by "S".

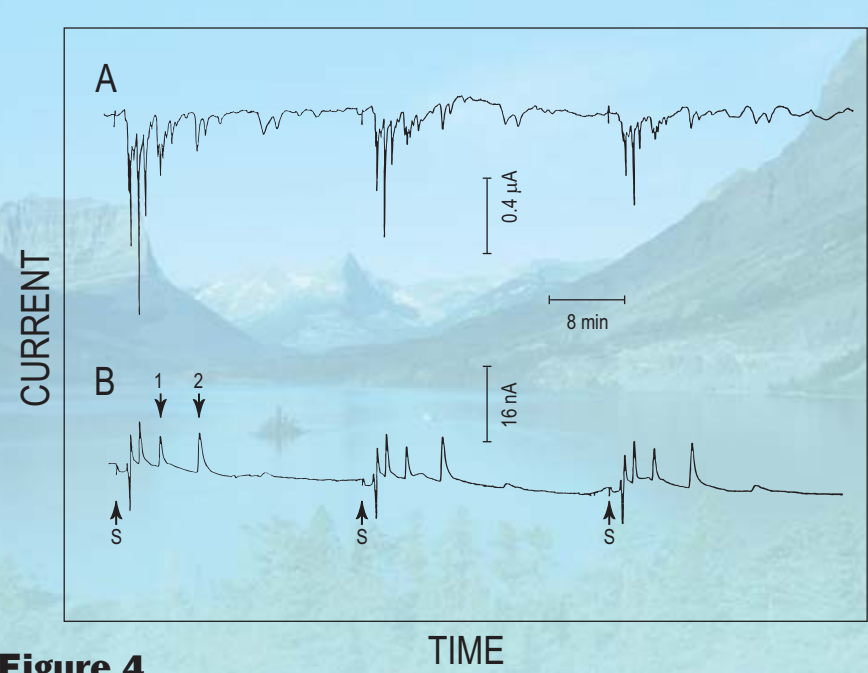


Figure 4

Table 1. Phenol Contaminants in Environmental Samples			
Sample	Compounds Detected	Concentrations Measured Using Tyr-CPE (ppm)	Concentrations Measured Using CPE (ppm)
Leachate 1 (EPA Region III)	p-cresol	24	21
	phenol	4	4
Leachate 3 (EPA Region II)	phenol	29	21
	p-acetamidophenol	ND	6
	p-nitrophenol	ND	4
Sludge	p-methoxyphenol	14	NM
	p-cresol	5	NM
	phenol	4	NM

ND, not detected using the specified electrode detector; NM, not measured.

Table 1 shows the phenol compounds identified and measured in contaminated leachate samples (1 and 3) and a sludge sample from Region III using both the CPE and Tyr-CPE detectors. The primary contaminants in the leachate samples were p-cresol and phenol and were observed with both the Tyr-CPE and CPE detectors. Minor contaminants present in Leachate 2 (p-acetamidophenol and p-nitrophenol) were only observed by the CPE detector. The combined phenols measured in Leachate 1 using both the Tyr-CPE detector (28 ppm) and CPE detector (25 ppm) corresponded well with the total phenols value reported to us by Region III for this sample (23 ppm) measured using the optical method for total phenols (3). Contaminants identified in the sludge sample analyzed using only the Tyr-CPE detector included p-methoxyphenol, p-cresol, and phenol.

**Figure 5. Comparison of the stability of the Tyr-CPE (A) and CPE (B) response to p-cresol in extracts of soil that had been spiked with the nine phenol mixture described in Fig. 4.** After ten consecutive injections of the extract over a 2 hr period, the CPE detector retained only 35% of its original response to p-cresol (Fig. 5B) whereas the Tyr-CPE biosensor detector retained 95% of its response (Fig. 5A). The decrease in the CPE response for the oxidation of phenols after sequential injections is not unexpected. Oxidation of these compounds has been reported to result in the condensation of quinones into polymeric films which passivate the electrode surface. In the case of the Tyr-CPE, the quinones formed by enzymatically catalyzed oxidation are rapidly reduced at the electrode surface. The rapid removal of quinones as well as the low operating potential may account for the stability of the Tyr-CPE compared to the CPE detector.

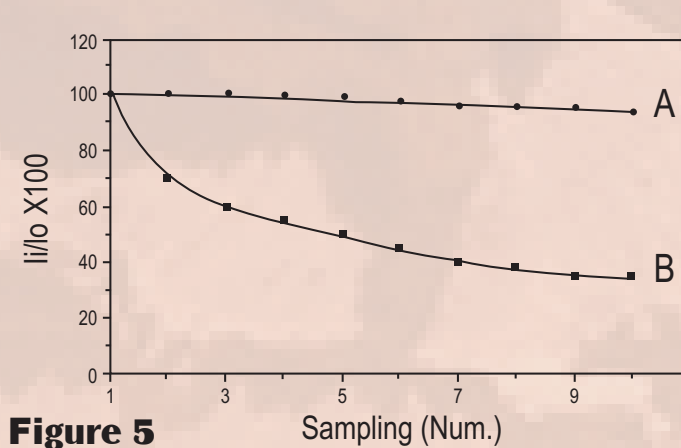


Figure 5

## 5. SUMMARY

The potential value of tyrosinase enzyme based methods for environmental screening and monitoring applications has been recognized and reported for matrices such as ground water, river water, and industrial waste water. In this report we demonstrate an HPLC system with a Tyr-CPE biosensor detector for the analysis of several contaminated environmental matrices including aqueous leachates and soil/sludge extracts. This method shows a concentration range which is applicable to screening these contaminated samples for the presence of phenol and para-substituted phenols and was not adversely affected by several environmental matrices. In addition, although this system shows a concentration range and detection limit comparable to a standard colorimetric screening method used for total phenols analysis, it does not require the complex distillation steps required for the optical method. As a result, the herein reported technique shows the potential to be developed as a field analytical method for routine on-site monitoring of phenolic compounds present in a variety of environmental samples. Current work is underway in our lab to develop this system with a removable tyrosinase biosensor which would allow for the determination of individual phenols prior to the field analysis of total phenols.

## 6. REFERENCES

- Kim R. Rogers, "Biosensors for Environmental Applications," *Biosensors Bioelectronics*, 10, 533-541, (1995).
- Kim R. Rogers, James Y. Becker, Joseph Wang and Fang Lu, "Determination of Phenols in Environmentally Relevant Matrices with the use of Liquid Chromatography with an Enzyme Electrode Detector", *Field Anal. Chem. Tech.*, 3, 161-169, (1999).

## 7. NOTICE

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